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Therapeutic Strategies for Eliminating Biofilm Produced by Staphylococcus Aureus Isolated from Catheter by Exposing to Radiation Emitted from Radioactive Sources (In Vitro)

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Abstract

This study aimed to evaluate the effectiveness of Sr90 radiation in eliminating biofilm production and inhibiting the growth of Staphylococcus aureus, a Gram-positive, facultative anaerobic bacterium responsible for infections such as pneumonia, sepsis, and bacteremia, whose virulence is enhanced by biofilm formation. A cross-sectional descriptive design combined with a case–control analytical approach was employed, with bacterial isolates obtained from catheters and other clinical samples of patients in Baghdad hospitals during 2023–2024. Biofilm-producing S. aureus isolates were exposed to varying doses of Sr90 radiation, with and without aluminum shielding, and their biofilm activity was assessed using Congo-Red Agar before and after exposure. Results demonstrated that all isolates (100%) exhibited biofilm production prior to treatment; however, after exposure to Sr90, biofilm production was completely inhibited (0%), as indicated by red colony coloration on Congo-Red Agar. Furthermore, Sr90 exposure produced 100% bactericidal activity across increasing doses and time intervals, whether aluminum shielding was applied or not. These findings provide evidence that Sr90 radiation effectively disrupts biofilm production and eradicates S. aureus, highlighting its potential as an innovative therapeutic strategy for managing biofilm-associated infections.

INTRODUCTION

Staphylococci are Gram-positive bacteria that are non-motile, non-spore forming, and facultative anaerobes capable of growing under aerobic respiration. They are considered among the most clinically important pathogens due to their ability to infect both immunocompromised patients in hospital settings and immunocompetent individuals in the community (Lowy, 1998). *Staphylococcus aureus*, in particular, has been identified as a leading cause of diseases ranging from superficial skin infections to more severe and life-threatening conditions such as pneumonia, sepsis, and bacteremia (Tong et al., 2015; Klevens et al., 2007). The increasing prevalence of antimicrobial resistance in *S. aureus*, including methicillin-

resistant *S. aureus* (MRSA), has further intensified the clinical significance of this pathogen and underscores the urgent need for alternative therapeutic strategies. One of the key challenges in controlling *S. aureus* infections is its capacity to form biofilms, which provide structural and functional protection against conventional antimicrobial agents. Biofilms are structured microbial communities encased in a self-produced extracellular polymeric matrix that adhere to biotic and abiotic surfaces, including indwelling medical devices. Their presence substantially complicates treatment by reducing antibiotic penetration, facilitating horizontal gene transfer of resistance, and shielding bacteria from host immune responses (Hall-Stoodley et al., 2004; Flemming & Wingender, 2010).

Natural products have been widely studied as potential anti-biofilm agents due to their biocompatibility and broad-spectrum activity. Plant-derived essential oils, phenolic compounds, and phytochemicals have been reported to exhibit promising biofilm inhibitory and disruptive effects (Nostro et al., 2007; Burt, 2004). For example, phenolic acids such as gallic acid, caffeic acid, and ferulic acid have been demonstrated to interfere with microbial adhesion and quorum sensing pathways, thereby reducing biofilm formation (Borges et al., 2013; Lou et al., 2011; Daglia, 2012). These natural compounds are considered safer and more sustainable compared to synthetic antimicrobials, particularly because they are less likely to induce resistance when used appropriately. Historically, the search for bioactive plant-derived compounds has been a cornerstone of antimicrobial drug discovery, dating back to the mid-20th century when the clinical utility of antibiotics was first realized (Fleming, 1929; Laxminarayan et al., 2013). However, with the rise of multidrug-resistant bacteria, the focus has shifted towards repurposing natural products as anti-biofilm therapeutics to overcome the limitations of conventional antibiotics.

The process of biofilm development in *S. aureus* involves four distinct phases: initial attachment to a surface, adherence and production of extracellular polymeric substances, biofilm maturation with the formation of three-dimensional structures, and eventual dispersal of cells to colonize new niches (Hall-Stoodley et al., 2004; Flemming & Wingender, 2010). Each stage of biofilm development is regulated by a complex network of signaling pathways, transcriptional regulators, and environmental cues. Quorum sensing (QS), a cell-to-cell communication mechanism, plays a central role in biofilm formation by coordinating the expression of genes involved in adhesion, extracellular matrix production, and virulence factor release. QS relies on the detection of signaling molecules, often referred to as autoinducers, which accumulate in the extracellular environment and trigger gene expression once threshold concentrations are reached (Waters & Bassler, 2005). In *S. aureus*, the accessory gene regulator (Agr) system represents the primary QS pathway, which modulates biofilm formation and virulence. The Agr system regulates the expression of adhesins during early stages of colonization and induces proteases and toxins during biofilm dispersal, thereby balancing the pathogen's persistence and invasiveness (Novick & Geisinger, 2008; Xu et al., 2022; Ma et al., 2022).

Developing novel anti-biofilm strategies has therefore focused on targeting QS pathways and interfering with bacterial adhesion and extracellular polymer production (Juszczuk-Kubiak, 2004; Iaconis et al., 2024). Natural products, including plant extracts and photochemicals, have shown promise in suppressing microbial cell adhesion, deactivating polymer synthesis, reducing virulence factor production, and obstructing QS signaling (Koo et al., 2017; Tan et al., 2020). Such strategies hold potential not only for treating established biofilm-associated infections but also for preventing biofilm formation on medical devices such as catheters, prosthetics, and implants. Yet, despite the potential, translating these natural anti-biofilm agents into clinical use remains challenging due to issues of

stability, bioavailability, and variability in active compound concentrations. Rigorous clinical trials and standardized formulations are required to bridge the gap between laboratory findings and practical medical applications (Richesson & Krischer, 2007; Ogbeta et al., 2023; Mentz et al., 2016; Ezzelle et al., 2008).

Beyond natural compounds, novel approaches involving physical and chemical agents are being explored. Strontium-90 (Sr90), a radioactive isotope produced through nuclear fission, has gained attention as a potential therapeutic tool due to its unique radiobiological properties (Chakravarty & Dash, 2012; Van Tuyle et al., 2003). With a half-life of 28.8 years, Sr90 undergoes β− decay into yttrium-90, releasing a decay energy of approximately 0.546 MeV (Firestone & Shirley, 1996). Its medical applications have traditionally centered on radiotherapy, particularly in the treatment of bone cancer and ophthalmic conditions, as well as industrial uses in radioisotope thermoelectric generators. Recent experimental evidence suggests that Sr90 radiation may also disrupt bacterial biofilms by damaging microbial DNA, impairing quorum sensing signaling, and reducing the ability of *S. aureus* to maintain its extracellular matrix. Importantly, preliminary studies indicate that exposure of biofilm-forming *S. aureus* to Sr90 results in a complete loss of biofilm production, as evidenced by colorimetric changes on Congo-Red Agar assays. While this finding is promising, it raises critical questions regarding the safety, specificity, and practicality of deploying radioactive isotopes in antimicrobial therapy.

METHODS

This study employed a combined case–control and cross-sectional design to examine the effectiveness of Sr90 radiation in eliminating biofilm production and inhibiting the growth of *Staphylococcus aureus*. The descriptive component provided an overview of biofilm formation among bacterial isolates, while the analytical component compared outcomes between exposed and unexposed groups under controlled laboratory conditions. This design was selected to allow both documentation of the prevalence of biofilm-producing *S. aureus* isolates and evaluation of their response to radiation treatment.

The study population consisted of 100 *S. aureus* isolates obtained from patients in Baghdad hospitals during the period of 2023–2024. The isolates were derived primarily from catheter samples, although additional clinical specimens were included where available. Identification of the bacterial species was carried out through classical biochemical screening techniques to ensure accuracy and reliability. Only isolates confirmed as *S. aureus* were included in the analysis, while contaminated or inconclusive samples were excluded.

Data collection involved two main experimental procedures. The first focused on exposing the isolates to radiation emitted from Sr90 sources at varying doses and durations, both in the presence and absence of aluminum shielding. Bacterial suspensions were standardized to a 0.5 McFarland turbidity level in sterile saline to ensure consistent bacterial density across all trials. Each sample was then subjected to Sr90 exposure under controlled laboratory conditions. Replicate assays were performed to strengthen reliability and reproducibility of results. After exposure, bacterial viability was assessed through growth on Tryptone Soy Agar, and the bactericidal effect was quantified using the ratio of colony counts between treated and control groups.

The second procedure assessed biofilm production before and after radiation exposure using Congo-Red Agar (CRA). This medium was prepared with brain–heart infusion broth, sucrose, agar, and Congo red dye, which enabled color-based differentiation of biofilm-forming colonies. Black, dry colonies were indicative of biofilm production, whereas red colonies signified absence of biofilm. The CRA test was applied both to isolates prior to treatment and to those subjected to radiation to

evaluate the impact of Sr90 on biofilm-forming ability. Observations were recorded after 24 to 48 hours of incubation at 37°C.

Data analysis was primarily descriptive, focusing on percentage differences between groups before and after radiation exposure. The proportion of isolates producing biofilm was calculated for baseline and post-treatment conditions, while bactericidal activity was reported as a percentage reduction in viable colony counts. Where relevant, results were compared across different doses and exposure times, as well as between conditions with and without aluminum shielding, to assess the consistency and robustness of the observed effects.

Ethical considerations were addressed by obtaining approval from the College of Ibn Sina University of Medical and Pharmaceutical Sciences. All procedures were conducted in accordance with institutional biosafety guidelines for handling clinical isolates and radioactive materials. Patient confidentiality was preserved, as only bacterial isolates were used and no personal identifying information was collected. The study is limited by its in vitro design, which does not fully replicate the complexity of host environments or clinical infections. Nevertheless, the detailed experimental approach and standardized procedures ensure reproducibility and provide a strong foundation for future research, including in vivo validation and potential translational applications.

RESULTS AND DISCUSSION

Study design

Case-Control study design depending in it research for analytical study design with Cross-Sectional for descriptive study design.

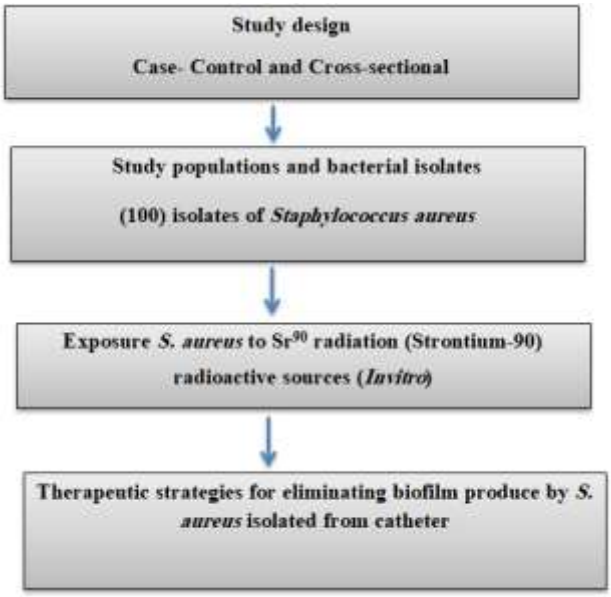

Figure 1. Plan of study design of this explore

Study populations and Bacterial isolates

A overall of assemblage of specimens (100) S. *aureus* for patients that were assumptive in Baghdad hospital through 2023/2024 which diagnosis via classical biochemical screening based.

Therapeutic strategies for exposure *S. aureus* to Strontium (Sr^{90}) radioactive sources in different doses

S. aureus grow was completed depending to [19] with many alterations, implanted within Nutrient stock at 37° C for 24 h, subsequently throw away at 5000 rpm until

10 min. The pellet was hanging of sterile naturalistic saline and compare with MacCfrland 0.5, subsequently exhibition 1 ml of hanged to rays released of Sr^{90} with comparison of dominance (wanting exposure to rays), every round was done in replicate and injected in Trypton soy agar.

The equation of attribution of death:

$$\text{Ratio of death \%} = \frac{\text{Dominance – patronized}}{\text{Dominance}} * 100$$

Therapeutic strategies for eliminate biofilm produced by S. *aureus* isolated from catheter by exposing to Sr^{90}

Congo Red process, the media formative of BHIB (37 g/ l), sucrose 5g/ l), agar numeral 1 (10 g/ l) and Congo red dye (0.8 gm/ l). Congo red dye was all set intensive liquid sol and autoclaved at 121 ^{o}C for 15 minutes. Thereafter, it was added up to autoclaved Brain heart infusion agar with each other with sucrose at 55 ^{o}C. Paten was grown with test organism and brood at 37 ^{o}C for 24 to 48 hr. aerobically. Black colonies to a dry crystalline tenacity particular biofilm output [20].

RESULTS AND DISCUSSION

The disintegration of the extra-polysaccharide is of specific connection for anti-biofilm mensuration. Thus far, different factors have been utilized to eliminate elementary and mingled species biofilms, originally via degenerating auto-produced adhesions, nucleic acids and polysaccharides.

The results in table (1) and figure (2) show biofilm production from *S. aureus* bacteria before and after exposure to radiation emitted from the Sr^{90} radioactive source (Strotium-^{90}). The bacteria were biofilm producers before (100%) exposure to radiation and after exposure to radiation. All bacteria lost biofilm production by (0%) when grown on the medium Congo-Red Agar (CRA) which changed the color of the bacterial colonies to red. This is an indication and evidence of their loss of biofilm production compared to the control before exposure to radiation emitted by Sr^{90}, whose colonies were colored. Dark black on Congo-Red Agar medium. Thus, it was proven that the radiation emitted by Sr^{90} is very effective and powerful in eliminating the biofilm of bacteria isolated from heart and urinary tract catheterization devices and isolated from patients after their catheterization procedure.

Table 1. Biofilm production from *S. aureus* before and after radiation

No.	Biofilm production before radiation	Percentage	Biofilm production after radiation	Percentage
1	+	100%	-	0%
2	+	100%	-	0%
3	+	100%	-	0%
4	+	100%	-	0%
5	+	100%	-	0%
6	+	100%	-	0%
7	+	100%	-	0%
8	+	100%	-	0%
9	+	100%	-	0%
10	+	100%	-	0%
11	+	100%	-	0%
12	+	100%	-	0%
13	+	100%	-	0%
14	+	100%	-	0%

15	+	100%	-	0%
16	+	100%	-	0%
17	+	100%	-	0%
18	+	100%	-	0%
19	+	100%	-	0%
20	+	100%	-	0%
21	+	100%	-	0%
22	+	100%	-	0%
23	+	100%	-	0%
Control (++++) Black colony		(+) : Positive production/ Black colony (-) : Negative production/ Red colony		

A previous study by [22] display Nerolidol was locate to repress *S. aureus* biofilm via more seventy percentage at concentricity extending of one to four mg/ml.

A preceding study by exhibit Alkaloids, aromatic acids are naturalistic components that anti-biofilm efficiency contra *S. aureus* like, the alkaloid sinomenine able essentially up regulate *agrA* and down-regulate *icaA* level.

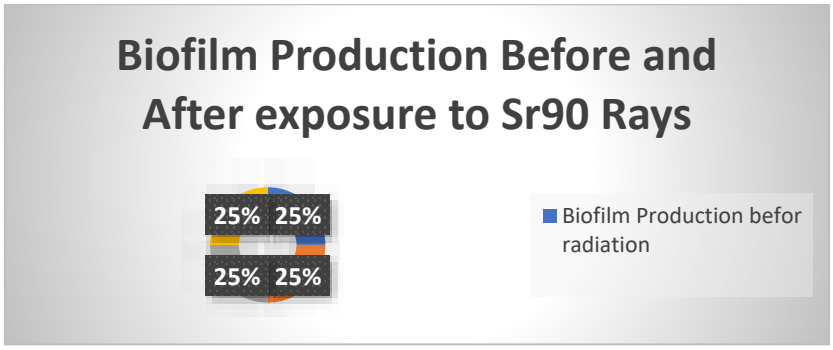

Figure 2. Biofilm production from *S. aureus* before and after exposure to Sr^{9} radiation

A antecedent study by revelation restricted therapeutic chosen for *S. aureus* recognizing efficient incorporation supplies an substitutional for infection therapy. Like, aside from possess considerable biological effectiveness, curcumin-founded metal complicated induce the bioavailability of curcumin. At the concentricity of one-hundred µM, curcumin suppressed *S. aureus* biofilm formulation fifty-six percentage whilst oxovanadium complicated of curcumin has a safely sturdy impact eithy-two percentage that might stay in the interaction together impact of complicated technicality have suppression and antibacterial technicality.

Figure 3. A- *S. aureus* production biofilm before exposure to Sr^{90} radioactive sources. B- *S. aureus* production biofilm after exposure to Sr^{90} radioactive sources

A past study by show the extractor of methanol from Hemidesmus indicus root that interaction together the anti-biofilm effectiveness of AML and CL contra Methicillin Resistance *S. aureus*. Furthermore, in the renal and liver of Methicillin Resistance *S. aureus* -infective mouse, the aggregates safely minimized microbial carriage, sickness effectiveness effect and gram-positive place.

Results of therapeutic strategies in table (2) for killing *S. aureus* exposed to Sr^{90} radiation wanting alaminium in activity 10 µci, percentage of humicides 100 % through doses 3.284235 through 1 hr.; 6.568471 in 2 hr.; 9.852705 in 3 hr. Also, exposed to Sr^{90} radiation wanting alaminium of activity 10 µci, percentage of humicide 100 % in doses 3.284235 through 1hr; 6.568471 through 2 hr.; 9.852705 through 3 hr. Also, exposed to Sr^{90} radiation to alaminium in activity 10 µci, percentage of humicides 100% via doses 5.2076*10-5 through 1hr.; 1.04153*10-4 through 2 hr. and 1.56228*10-3 through 3 hr. as shown in table (2).

Table 2. Proportion of homicides of *S. aureus* exposure to Sr^{90} radioactive sources.

No.	Activity	Doses in 1hr./ msv	Proportion of assassination	Doses in 2hr./ msv	Proportion of assassination	Doses in 3hr./ msv	Proportion of assassination
Sr^{90} Without Alaminium	10 µci	3.284235	100 %	6.568471	100 %	9.852705	100 %
Sr^{90} With Alaminium	10 µci	5.2076*10-5	100 %	$1.04153*10^{-4}$	100 %	$1.56228*10^{-3}$	100 %
Control = 250 Colony							

A anterior study by demonstration a fatal impact on *Pseudomonas* through displayed to rays on various times through (1,2,3) hr. with diverse doses by cobalt with impact 1 µci and 10 µci in the existence and non-attendance of aluminium with the use of strontium in the existence and non-attendance of aluminium. The killing rate of *P. aeruginosa* by cobalt wanting aluminium was 78% with in the existence of aluminium 100%. In rapprochement, the death average of *Pseudomonas* with efficient wanting aluminium was 100%; in the existence of aluminium 98%, the death average of *Pseudomonas* wanting aluminium was 83% and in the existence of aluminium 96% rapprochement with control.

Discussion

The findings of this study clearly demonstrate that exposure to Sr90 radiation eliminated biofilm production of *Staphylococcus aureus* and resulted in a complete bactericidal effect under different experimental conditions. While the results provide strong laboratory evidence, their true value lies in how they contribute to the broader understanding of biofilm control and how they may inform alternative therapeutic strategies in clinical microbiology. Biofilm-associated infections continue to present significant challenges due to their resistance to antibiotics and host immune

responses, and thus, any potential approach that shows strong anti-biofilm activity warrants careful consideration.

In comparing the present results with earlier studies, it is evident that Sr90 radiation exhibits a unique mode of action compared with conventional or natural antimicrobials. Previous research on plant-derived compounds such as phenolic acids, alkaloids, or curcumin derivatives indicated partial suppression of biofilm activity, often ranging between 50 to 80 percent inhibition under specific concentrations. In contrast, the current results show a complete absence of biofilm production after Sr90 exposure, a finding that suggests stronger and more consistent disruption of bacterial structures. Although other physical interventions such as cobalt irradiation have been reported to reduce microbial viability, the total elimination of biofilm observed here highlights the potential superiority of Sr90 in this context.

The mechanisms behind these effects are likely multifaceted. Radiation from Sr90 emits beta particles that can penetrate microbial cells, producing double-stranded DNA breaks and damaging other cellular macromolecules. Such damage may interfere with quorum sensing pathways that regulate biofilm formation and also disrupt the synthesis of extracellular polymeric substances. Without these structures, *S. aureus* cannot maintain stable biofilm communities, leaving cells more vulnerable to death. This mechanistic explanation aligns with the observed color change on Congo-Red Agar, where colonies lost the black appearance typical of biofilm producers and became red, indicating absence of extracellular matrix formation. Understanding these mechanisms is crucial not only for explaining the current findings but also for guiding the design of future interventions that target similar pathways.

The implications for clinical practice are significant, particularly in the management of device-related infections. Catheter-associated infections caused by *S. aureus* are notoriously difficult to treat because biofilms protect bacteria from antibiotics and immune clearance. The ability of Sr90 radiation to eliminate biofilms suggests that it could serve as a basis for novel strategies to sterilize medical devices or to treat persistent infections resistant to standard therapy. This is especially relevant in an era where antibiotic resistance continues to rise and available treatment options are limited. However, these potential applications must be weighed carefully against the inherent risks of using radioactive materials in medical settings. Safety protocols, dosage optimization, and controlled delivery systems would be essential to minimize collateral damage to human tissues.

A broader consideration is the practicality of implementing radiation-based antimicrobial therapies. While laboratory settings allow for controlled and safe exposure of bacterial cultures, translating this into patient care raises multiple challenges. The long half-life of Sr90, its radiotoxic potential, and issues of disposal and containment could limit its use outside specialized facilities. Moreover, regulatory and ethical concerns would require robust evidence from animal studies and clinical trials before any application could be considered. These barriers should not be underestimated, yet they should also not overshadow the novelty of the findings, which add to the growing body of knowledge on unconventional approaches to biofilm eradication.

Despite the promising results, it is essential to recognize the limitations of the present study. All experiments were conducted in vitro, and the controlled environment of laboratory assays does not fully replicate the complexity of human infections, where host immune responses, tissue microenvironments, and microbial diversity influence outcomes. The exclusive focus on *S. aureus* also limits generalizability, as biofilm behaviors differ among bacterial species. Furthermore, the

study did not investigate the possible development of resistance mechanisms against radiation exposure, an area that may warrant exploration in long-term or repeated exposure models. Acknowledging these limitations helps prevent overgeneralization and provides a balanced perspective on the significance of the results.

Future research should therefore aim to validate these findings in more complex systems. Animal models could provide insights into both efficacy and safety, while experiments involving mixed-species biofilms might clarify whether Sr90 remains effective in the polymicrobial environments typical of clinical infections. It would also be valuable to explore whether lower doses or shorter exposure times could achieve comparable effects, as this would reduce potential risks associated with radiation. Additionally, combining radiation with other therapies, such as antibiotics or natural compounds, could offer synergistic effects that enhance antimicrobial efficacy while mitigating drawbacks.

CONCLUSION

Production of biofilm from S. aureus lower after exposure to Sr90 without alminium indicate by the color of culture become red compared with control is black colony.

Ethical approval

All examination protocols were confirmed by the College of Ibn Sina Universty of Medical and Pharmaceuticals Sciences. All screening was achieved following the confirmed guidelines.

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There was no financial disclosure.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Researcher Dr. Nebras Rada Mohammed PhD. in Biotechnology with a Genetic Engineering, Molecular Genetics and Protein Engineering, a scientist, expert, researcher, creator, inventor, writer, written and author, editor-in-chief of the Journal of Articles and Inventions in the American Goidi Journal, teaching, lecturer at the University College of Al-Turath University college, a Bachelor's degree in Microbiology and a Master's degree in Molecular Biology in Microbiology from Al-Mustansiriya University, an arbitrator, international resident and consultant In medical laboratories, an expert in medical laboratories and a holder of the title of a scientist project, an arbitrator, a distinguished publisher, a silver supporter of scientific platforms, a chairman of a committee in a scientific society, receiving accolades from international intellectual property, the Best Arab Woman Award 2020, also the Best Community Personality Award, the Best Research Award 2019, also the Best Research Award 2020 and an American Award For the invention of 2020 by the American Goidi the World Investment Commission in America, holds the title of the best distinguished inventor in the world by the World Investment Commission in America and holds the first places in the world for inventions presented in the world from the American Goidi, the world investment commission in America. The Edison Prize, The Pascal Prize, The creativity award, the scientific medal and the Everest medal for innovation, creativity for inventions from USA.

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